

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claim 1 (currently amended): A polysaccharide-protein conjugate or an oligosaccharide-protein conjugate comprising:

a de-N-acetylated ~~an N-propionated~~ polysaccharide or a de-N-acetylated N-
propionated-oligosaccharide covalently attached to ~~directly coupled to~~ a protein via a β -
propionamido linkage,

wherein said de-N-acetylated polysaccharide or said de-N-acetylated
oligosaccharide is derived from bacterial, yeast or cancer cell surface or capsular polysaccharide
or oligosaccharide, naturally or synthetically obtained, through β position sites of one or more
propionate moieties of the N-propionated polysaccharide or N-propionated oligosaccharide;

~~wherein the N-propionated polysaccharide or N-propionated oligosaccharide~~
~~directly coupled to the protein~~ the polysaccharide-protein conjugate or the oligosaccharide-
protein conjugate ~~elicits protective antibodies reactive with the N-propionated-polysaccharide or~~
~~N-propionated-the oligosaccharide[;];~~

wherein the degree of de-N-acetylation is N-propionated polysaccharide or N-
propionated oligosaccharide is de N acetylated and N acryloylated; wherein at least 50% of the
N-propionated polysaccharide or oligosaccharide is de N acetylated; and

wherein the protein is [[a]]bacterial protein or [[a]]synthetic protein-containing
comprising a lysine residue or a cysteine residue[[s]].

Claims 2-3 (canceled).

Claim 4 (currently amended): The conjugate according to claim 1 wherein the

polysaccharide or the oligosaccharide is ~~derived~~ ~~obtained~~ from *Escherichia coli*, Meningococcus, Pneumococcus, Streptococcus, Neisseria, Salmonella, Klebsiella, or Pseudomonas.

Claim 5 (currently amended): The conjugate according to claim 1 wherein the polysaccharide or the oligosaccharide is ~~derived~~ ~~obtained~~ from Group B *Streptococcus* selected from the group consisting of type Ia, type Ib, type II, type III, type V, type VIII, and combinations thereof.

Claim 6 (currently amended): The conjugate according to claim 4 wherein the polysaccharide or the oligosaccharide is derived from a Meningococcus group selected from the group consisting of group B, group C, group Y, group W135, and combinations thereof.

Claim 7 (currently amended): The conjugate according to claim 4 wherein the polysaccharide or the oligosaccharide is derived from *E. coli* K1, *E. coli* K92, Pneumococcus type 4, Pneumococcus type 14, Streptococcus group A, Streptococcus group C, or combinations thereof.

Claim 8 (previously presented): The conjugate according to claim 1 wherein the protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, a *Neisseria meningitidis* outer membrane protein, pneumolysoid, C- β protein from group B *Streptococcus* and non-IgA-binding C- β protein from group B *Streptococcus*.

Claim 9 (previously presented): The conjugate according to claim 8 wherein the protein is recombinantly produced.

Claim 10 (previously presented): The conjugate according to claim 9 wherein the protein is recombinant *N. meningitidis* outer membrane protein.

Claim 11 (currently amended): The conjugate according to claim 1 wherein the polysaccharide or the oligosaccharide comprises a glycosaminoglycan.

Claim 12 (currently amended): The conjugate according to claim 1 wherein the polysaccharide or the oligosaccharide comprises glycosyl residues of a structural repeating unit having at least one free amino group or N-acyl group.

Claim 13 (previously presented): The conjugate according to claim 12 wherein the glycosyl residue is selected from the group consisting of glucosamine, galactosamine, mannosamine, fucosamine and sialic acid.

Claim 14 (currently amended): The conjugate according to claim 1 wherein the ~~N-propionated polysaccharide or N-propionated oligosaccharide is directly coupled to β -propionamido linkage~~ comprises a β -carbon attached to: (1) a side-chain nitrogen of ~~the an ϵ -free amino group of a~~ lysine residue of the protein, or (2) a ~~thiol group~~ sulfur of ~~[[a]]~~ the cysteine residue of the protein.

Claim 15 (currently amended): The conjugate according to claim 1 wherein the polysaccharide or the oligosaccharide is obtained from Group B *Streptococcus* type III, and wherein the protein is tetanus toxoid.

Claim 16 (currently amended): A polysaccharide-protein conjugate or an oligosaccharide-protein conjugate ~~comprising an N-propionated polysaccharide or N-propionated oligosaccharide directly coupled to a protein through β position sites of one or more propionate moieties of the N-propionated polysaccharide or N-propionated oligosaccharide; wherein the conjugate elicits protective antibodies reactive with the N-propionated polysaccharide or N-propionated oligosaccharide, wherein said conjugate is produced by a method comprising:~~

A) de-N-acetylating ~~an isolated a~~ bacterial, yeast or cancer cell surface or capsular polysaccharide or oligosaccharide, naturally or synthetically obtained, by at least 50% using a

de-N-acetylating reagent to form a de-N-acetylated polysaccharide or a de-N-acetylated oligosaccharide, ~~wherein at least 50% of the N-propionated polysaccharide or N-propionated oligosaccharide is de-N-acetylated;~~

B) N-acryloylating the de-N-acetylated polysaccharide or the de-N-acetylated oligosaccharide with an acryloylating reagent to form an ~~N-propionated~~ N-acryloylated polysaccharide or an ~~N-propionated~~ N-acryloylated oligosaccharide, and

C) ~~directly coupling through β position sites of one or more propionate moieties of the N-propionated~~ reacting the N-acryloylated polysaccharide or the N-propionated-N-acryloylated oligosaccharide with protein to form a β -propionamido linkage,

wherein the protein is [[a]] bacterial protein or [[a]] synthetic protein containing comprising a lysine residue or a cysteine residue[[s]], and to form the polysaccharide-protein conjugate or the oligosaccharide-protein conjugate.

wherein the polysaccharide-protein conjugate or the oligosaccharide-protein conjugate elicits protective antibodies reactive with the polysaccharide or the oligosaccharide.

Claim 17 (canceled).

Claim 18 (currently amended): The conjugate according to claim 16 wherein the N-acryloylated polysaccharide or the N-acryloylated oligosaccharide is reacted with the protein coupling is conducted at a pH of about 7.0.

Claim 19 (currently amended): The conjugate according to claim 16 wherein the N-acryloylated polysaccharide or the N-acryloylated oligosaccharide is reacted with the protein coupling is conducted at a pH above 9.

Claim 20 (currently amended): The conjugate according to claim 16 wherein the N-acryloylated polysaccharide or the N-acryloylated oligosaccharide is reacted with the protein

~~coupling is conducted~~ in a reagent selected from the group consisting of phosphate buffer, bicarbonate buffer, and borate buffer.

Claim 21 (previously presented): The conjugate according to claim 16 wherein the de-N-acetylating reagent is a base or an enzyme and the acryloylating reagent is selected from the group consisting of N-acryloyl chloride, acryloyl anhydride, acrylic acid and a dehydrating agent.

Claim 22 (previously presented): A pharmaceutical composition comprising the conjugate according to any one of claim 1 and claim 16 and a pharmaceutically acceptable carrier.

Claim 23 (original): The pharmaceutical composition according to claim 22 further comprising an adjuvant.

Claim 24 (original): The pharmaceutical composition according to claim 23 wherein the adjuvant is selected from the group consisting of alum and stearyl tyrosine.

Claim 25 (currently amended): The pharmaceutical composition according to claim 22 further comprising a second immunogenic component, said second immunogenic component selected from the group of immunogens consisting of diphtheria-tetanus-pertussis (DTP), diphtheria-tetanus-acellular pertussis (DTaP), tetanus-diphtheria (Td), diphtheria-tetanus-acellular pertussis-*Haemophilus influenzae* type [[B]]_b (DTaP-Hib), diphtheria-tetanus-acellular pertussis-inactivated poliovirus-*Haemophilus influenzae* type [[B]]_b (DTaP-IPV-Hib), and combinations thereof.

Claim 26 (currently amended): An immunogen comprising the conjugate[[s]] according to any one of claim 1 and claim 16, wherein said immunogen elicits an immune response specific to the polysaccharide or the oligosaccharide~~an N-propionated polysaccharide~~

~~specific or an N-propionated oligosaccharide-specific immune response.~~

Claim 27 (currently amended): The immunogen according to claim 26, wherein the immune response is generation of an immunoglobulin specific to the polysaccharide or the oligosaccharide~~N-propionated polysaccharide-specific or an N-propionated oligosaccharide-specific immunoglobulin.~~

Claim 28 (original): The immunogen according to claim 27 wherein the immunoglobulin is IgG, IgM, IgA or combinations thereof.

Claim 29 (withdrawn): A method of making a β -propionamido-linked polysaccharide-protein conjugate or a β -propionamido-linked oligosaccharide-protein conjugate comprising:

A) de-N-acetylating a polysaccharide or an oligosaccharide using a de-N-acetylating reagent to form a de-N-acetylated polysaccharide or de-N-acetylated oligosaccharide,

B) N-acryloylating the de-N-acetylated polysaccharide or de-N-acetylated oligosaccharide with an acryloylating reagent to form a β -propionated polysaccharide or a β -propionated oligosaccharide, and

C) directly conjugating the β -propionated polysaccharide or the β -propionamido oligosaccharide to a protein to form the β -propionamido-linked polysaccharide-protein or β -propionamido-linked oligosaccharide-protein conjugate conjugate.

Claim 30 (withdrawn): The method of claim 29, wherein the de-N-acetylating reagent is a base or enzyme.

Claim 31 (withdrawn): The method of claim 29 wherein the de-N-acetylating reagent is selected from the group consisting of NaOH, KOH and LiOH.

Claim 32 (withdrawn): The method of claim 29, wherein the acryloylating reagent is

selected from the group consisting of acryloyl chloride, acryloyl anhydride, acrylic acid and a dehydrating agent.

Claim 33 (withdrawn): The method of claim 29, wherein the polysaccharide or oligosaccharide is obtained from bacteria, yeast, cancer cells or is chemically synthesized.

Claim 34 (withdrawn): The method of claim 29 wherein the polysaccharide or oligosaccharide is obtained from *Escherichia coli*, Meningococcus, Pneumococcus, Streptococcus, Neisseria, Salmonella, Klebsiella, or Pseudomonas.

Claim 35 (withdrawn): The method of claim 29 wherein the protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, a neisserial outer membrane protein, pneumolysoid, C- β protein from group B Streptococcus and non-IgA binding C- β protein from group B *Streptococcus*.

Claim 36 (withdrawn): The method of claim 35, wherein the protein is recombinantly produced.

Claim 37 (currently amended): A vaccine comprising the conjugate according to any one of claim 1 and claim 16, wherein said vaccine provides protective immunity against at least one member of a genus of an organism from which the polysaccharide or the oligosaccharide component of the polysaccharide-protein conjugate or oligosaccharide-protein conjugate was obtained.

Claim 38 (canceled).

Claim 39 (currently amended): The vaccine according to claim ~~[[38]]~~39 wherein the bacteria are selected from the group consisting of *Escherichia coli*, Meningococcus, Pneumococcus, Streptococcus, Neisseria, Salmonella, Klebsiella, and Pseudomonas.

Claim 40 (currently amended): The vaccine according to claim 37 further

comprising a second immunogen in combination with the polysaccharide-protein conjugate or the oligosaccharide-protein conjugate, said second immunogen selected from the group consisting of diphtheria-tetanus-pertussis (DTP), diphtheria-tetanus-acellular pertussis (DTaP), tetanus-diphtheria (Td), diphtheria-tetanus-acellular pertussis-*Haemophilus influenzae* type *[[B]]*_h (DTaP-Hib), diphtheria-tetanus-acellular pertussis-inactivated poliovirus-*Haemophilus influenzae* type *[[B]]*_h (DTaP-IPV-Hib), and combinations thereof.

Claim 41 (withdrawn): A method of immunizing a mammal against a disease causing organism or disease causing cell comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.

Claim 42 (withdrawn): A method of immunizing a mammal against *Streptococcus pneumoniae* comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.

Claim 43 (withdrawn): A method of immunizing a mammal against Group B *Streptococcus* comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.

Claim 44 (withdrawn): A method of immunizing a mammal against Group B *Neisseria meningitidis* comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.

Claim 45 (withdrawn): A method of immunizing a mammal against Group C *Neisseria meningitidis* comprising administering to the mammal an immunizing amount of the vaccine according to claim 37.

Claim 46 (withdrawn): A method of immunizing a mammal against *Haemophilus influenzae* type B comprising administering to the mammal an immunizing amount of the

vaccine according to claim 37.

Claim 47 (withdrawn): A method of eliciting an antibody response to a polysaccharide or an oligosaccharide in a mammal comprising administering an effective amount of the conjugate according to any one of claim 1 and 16.

Claim 48 (withdrawn): An immunoglobulin or antigen-binding fragment thereof produced according to the method of claim 47.

Claim 49 (withdrawn): The immunoglobulin according to claim 48, selected from the group consisting of IgG antibody, IgM antibody, IgA antibody and combinations thereof.

Claim 50 (withdrawn): The immunoglobulin according to claim 49, wherein the antibody is an isolated IgG.

Claim 51 (withdrawn): An isolated antibody or antigen binding fragment thereof elicited in response to the β -propionamido-linked polysaccharide-protein conjugate or β -propionamido-linked oligosaccharide-protein conjugate according to any one of claim 1 and 16, said antibody or antigen fragment thereof specifically immunoreactive with N-propionated polysaccharide or N-propionated oligosaccharide and immunoreactive with a native N-acetylated polysaccharide from which the β -propionated polysaccharide or β -propionated oligosaccharide was obtained.

Claim 52 (withdrawn): The antibody or antigen binding fragment thereof according to claim 51 wherein the native N-acetylated polysaccharide is obtained from bacteria, yeast, cancer cells, or is chemically synthesized.

Claim 53 (withdrawn): The antibody or antigen binding fragment thereof according a claim 52 wherein the polysaccharide is obtained from *Escherichia coli*, Meningococcus, Pneumococcus, Streptococcus, Neisseria, Salmonella, Klebsiella, or

Pseudomonas.

Claim 54 (withdrawn): The antibody or antigen binding fragment thereof according to claim 51 wherein the antibody is recombinantly produced.

Claim 55 (withdrawn): A method of passive immunization against a disease causing organism or disease causing cells comprising administration of an effective amount of the immunoglobulin or antibody according to claim 48, said amount is sufficient to inhibit or kill the disease causing organism or disease causing cells.

Claim 56 (withdrawn): The method of passive immunization according to claim 55 wherein the immunoglobulin is an isolated IgG antibody or antigen binding fragment thereof.

Claim 57 (withdrawn): The method of passive immunization according to claim 55 wherein the immunoglobulin is an isolated IgM antibody or antigen binding fragment thereof.

Claim 58 (withdrawn): The method of passive immunization according to claim 55 wherein the immunoglobulin is an isolated IgA antibody or antigen binding fragment thereof.

Claim 59 (currently amended): The conjugate according to claim 1, wherein the β -propionamido linkage is formed by N-acryloylating the de-N-acetylated polysaccharide or the de-N-acetylated oligosaccharide to form an N-acryloylated polysaccharide or an N-acryloylated oligosaccharide, and reacting an acryloyl moiety of the N-acryloylated polysaccharide or the N-acryloylated oligosaccharide with the protein, wherein the degree of N-acryloylation is at least 95% N-acryloylated.

Claim 60 (currently amended): The conjugate according to claim 16, wherein the de-N-acetylated polysaccharide or the de-N-acetylated oligosaccharide ~~is at least 95% N-acryloylated by at least 95%.~~

Claim 61 (previously presented): The conjugate according to any one of claim 1 and

claim 16, wherein the bacterial protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, cholera toxin subunit B, *Neisseria meningitidis* outer membrane proteins, pneumolysoid, C- β protein from group B Streptococcus, *Pseudomonas aeruginosa* toxoid, and pertussis toxoid.

Claim 62 (withdrawn): A method of passive immunization against a disease causing organism or disease causing cells comprising administration of an effective amount of the immunoglobulin or antibody according to claim 51, said amount is sufficient to inhibit or kill the disease causing organism or disease causing cells.

Claim 63 (withdrawn): The method of passive immunization according to claim 62 wherein the immunoglobulin is an isolated IgG antibody or antigen binding fragment thereof.

Claim 64 (withdrawn): The method of passive immunization according to claim 62 wherein the immunoglobulin is an isolated IgM antibody or antigen binding fragment thereof.

Claim 65 (withdrawn): The method of passive immunization according to claim 62 wherein the immunoglobulin is an isolated IgA antibody or antigen binding fragment thereof.